

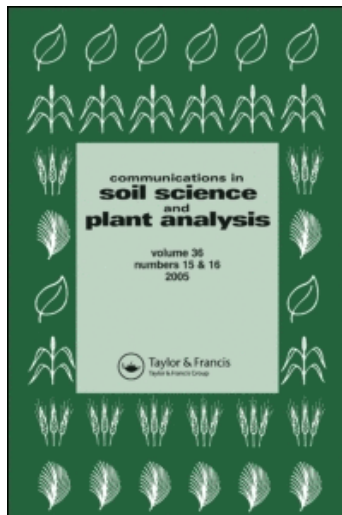
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Effect of Ethylenediaminetetraacetic Acid on Growth and Phytoremediative Ability of Two Wheat Varieties

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Chelating agents are commonly used to enhance the phytoremediative ability of plants. The type of chelating agent applied and the selection of plant species are important factors to consider for successful phytoremediation. This study investigates the effects of four different rates (0, 2, 4, 8 mmol kg⁻¹) of ethylenediaminetetraacetic acid (EDTA) on lead (Pb) dissolution, plant growth, and the ability of two spring wheat varieties (Auqab-2000 and Inqalab-91) to accumulate Pb from contaminated soils in a pot study. The results indicated that the addition of EDTA to the soil significantly increased the aqueous solubility of Pb and that wheat variety Inqalab-91 was more tolerant to Pb than Auqab-2000. Application of EDTA at 8 mmol kg⁻¹ resulted in biomass yield, photosynthetic rate, and transpiration rate significantly lower in Auqab-2000 than in Inqalab-91. Although EDTA enhanced the uptake of Pb by both wheat varieties, Auqab-2000 accumulated significantly more Pb in the shoots than Inqalab-91. The results of the present study suggest that under the conditions used in this experiment, EDTA at the highest dose was the best amendment for enhanced phytoextraction of Pb using wheat. High concentrations of Pb were found in leachates collected from the bottom of columns treated with EDTA. Application of EDTA in the column leaching experiment increased the concentration of Pb in leachate with increasing EDTA dosage (0–8 mmol kg⁻¹). These results suggest that EDTA addition for enhancing soil cleanup must be designed properly to minimize the uncontrolled release of metals from soils into groundwater.

Keywords Chelating agents, leaching, phytoremediation, wheat

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Introduction

The rapid industrialization and indiscriminate use of raw city effluent for irrigation in developing countries has led to accelerated addition of heavy metals onto soils. Among these metals, lead (Pb) is the most widespread and persistent metal pollutant in soils (Kumar et al. 1995). At excessive levels, Pb may cause severe growth reductions by disturbing chlorophyll formation, decreasing photosynthetic activity (Ruley et al. 2006), and loss of enzymatic activity (Wierzbicka et al. 2007). Moreover, its entry into the food chain from contaminated soils and waters may cause severe toxicity in animals and humans.

Because of the high cost and hazardous impacts on soil, microorganisms, and plants, engineering-based remediation strategies are becoming less attractive. As an alternative, phytoextraction has emerged as a promising and cost-effective remediation method (Salt, Smith, and Raskin 1998). Phytoextraction is the use of green plants for cleaning metal-contaminated sites. Phytoextraction can be broadly classified as either natural or chemical-assisted (Saifullah, Ghafoor, and Qadir 2009; Liu et al. 2008). In the natural phytoextraction approach, metal hyperaccumulating plant species are used (Kumar et al. 1995), because these plant species have the ability to accumulate metals in the aboveground biomass above a certain arbitrary threshold concentration, for example, on a dry-weight (DW) basis, 100 mg kg⁻¹ for cadmium (Cd); 1000 mg kg⁻¹ for arsenic (As), cobalt (Co), copper (Cu), Pb, or nickel (Ni); or more than 10,000 mg kg⁻¹ for manganese (Mn) or zinc (Zn) (Baker et al. 2000). The second phytoextraction approach makes use of high-biomass-producing plant species. Although these species lack the inherent ability to take up large amounts of heavy metals as characterized by hyperaccumulating species, they could accumulate large amounts of contaminants from soils that have been chemically treated with metal mobilizing agents (Meers et al. 2005).

Enhanced accumulation of metals by plant species with ethylenediaminetetraacetic acid (EDTA) treatment is attributed to many factors working either singly or in combination. These factors include (1) an increase in the concentration of available metals, (2) enhanced metal–EDTA complex movement to roots, (3) less binding of metal–EDTA complexes with the negatively charged cell wall constituents, (4) damage to physiological barriers in roots either due to greater concentration of metals or EDTA or metal–EDTA complexes, and (5) increased mobility of metals within the plant body when complexed with EDTA compared to free metal ions facilitating the translocation of metals from roots to shoots (Nowack, Schulin, and Robinson 2006; Xu et al. 2007). The damage to the root exclusion mechanism may be achieved by root pretreatment with hot water or chelating agents dissolved in hot water (Luo et al. 2006a), combined application of EDTA and nonionic surfactants (Gregorio et al. 2006), combined application of EDTA and ethylenediaminedisuccinic acid (EDDS) (Luo et al. 2006b), addition of EDTA to soils at high rates (Vassil et al. 1998), or transplanting seedlings instead of direct sowing of crops into contaminated soils (Wu, Hsu, and Cunningham 1999).

Plant species do vary in their accumulation response to heavy metals during EDTA-assisted phytoextraction (Shen et al. 2002; Chen, Li, and Shen 2004), which could be further enhanced by using the right combination of plant species and chelators (Salt et al. 1995). Differences in metal uptake have been reported not only at the species level, but significant variations have also been observed among varieties, cultivars, and genotypes (Kabata-Pendias and Pendias 2001; Huang et al. 1997). Chen, Li, and Shen (2004) reported that dicotyledonous species (mung bean, sunflower, cabbage, buckwheat, pea, and mustard) were more efficient in Pb uptake during EDTA-assisted phytoextraction than

monocotyledonous species (maize, sorghum, wheat, and barley). This greater phytoextraction capacity was attributed to heavier damage to physiological barriers by EDTA in dicotyledonous species than monocotyledonous species, which caused indiscriminate uptake of heavy metals (Luo et al. 2006a).

Although many researchers have investigated the comparative performance of various plant species for chelator-assisted metal uptake, little work has been done on the comparison of different varieties of the same species during chelator-assisted phytoextraction. To our knowledge, this is the first study that evaluates the performance of two varieties of the same species for chemical-assisted phytoextraction. These varieties of wheat differ in salt tolerance, growth conditions (Gulnaz et al. 1999; Afzal et al. 2006), and metal tolerance and as such could be expected to have differences in sensitivity of root exclusion mechanism to both EDTA and Pb levels. In addition, these wheat varieties were also commonly cultivated in both urban and periurban areas of the major cities of Pakistan, where these are irrigated with metal-rich raw city effluent and mainly harvested at bolting stage as fodder for large ruminants (Saifullah, Ghafoor, and Qadir 2009).

The present study was undertaken with three principle objectives: (1) to assess the effects of EDTA on growth, gas exchange, and ionic composition of two wheat varieties, (2) to determine the role of EDTA in inducing hyperaccumulation of Pb in two wheat varieties, and (3) to investigate the effect of EDTA on leaching of Pb from soils.

Materials and Methods

Soil Samples

Soil was collected from the 0- to 20-cm surface layer of an agricultural field irrigated with untreated city sewage located in a village, Kajlianwala, in Faisalabad, Pakistan. The soil was air dried, ground to pass through a 2-mm sieve, mixed thoroughly, and stored in plastic jars for analysis. Soil samples were analyzed for saturation paste pH (pH_s), pH of soil-to-water ratio ($\text{pH}_{1:2}$), saturation paste extract electrical conductivity (EC_e), organic matter (OM), lime contents (CaCO_3), soil texture, and cation exchange capacity (CEC) following the methods described by U.S. Salinity Laboratory Staff (1954) and Page, Miller, and Keeny (1982). Total concentrations of heavy metals (Cu, Mn, Pb, Zn) were determined following aqua regia digestion (McGrath and Cunliffe 1985), and ammonium bicarbonate–diethylenetriaminepentaacetic acid (AB-DTPA)–extractable metals (Cu, Mn, Pb, and Zn) were determined according to the method described by Soltanpour (1985). Total and AB-DTPA extractable metal concentration was determined using atomic absorption spectrometer (AAS; Thermo S-Series).

Artificial Contamination of Soil

For spiking the soil with Pb, soil was spread over a polyethylene sheet. The lead nitrate [$\text{Pb}(\text{NO}_3)_2$] salt solution (equal to 75% of soil saturation percentage) was sprayed over a thin layer of soil to obtain $500 \text{ mg Pb kg}^{-1}$ soil. Soil was thoroughly mixed to achieve uniformity in metal spiking. Soil was placed in large containers lined with polyethylene sheets, kept wet to near saturation, and allowed to equilibrate with periodic mixing for 3 weeks. The soil was air-dried, and the whole procedure was repeated for two additional equilibrium periods. At the end of the third equilibrium cycle, soil samples were taken to determine AB-DTPA–extractable Pb from the spiked soil (Table 1).

Table 1
Main physical and chemical properties of the soil

Parameters	Unit	Values
Textural class	—	Sandy clay loam (SCL)
Sand	%	49.10
Silt	%	24.50
Clay	%	26.40
pH _s ^a	—	8.16
pH _{1:2} ^b	—	8.67
EC _e	dS m ⁻¹	3.5
SAR	(mmol L ⁻¹) ^{1/2}	16.0
CEC	cmol _c kg ⁻¹	6.15
CaCO ₃	%	0.98
OM	%	0.82
Pb (before incubation)	mg kg ⁻¹	32.29(3.3) ^c
Pb (after incubation)	mg kg ⁻¹	^a 435.20(322.3) ^c
Zn	mg kg ⁻¹	^a 74.25(3.4) ^c
Cu	mg kg ⁻¹	^a 22.76(3.75) ^c
Mn	mg kg ⁻¹	^a 510.96(4.01) ^c

^apH by saturated soil paste extract.

^bpH by soil to water ratio (1:2).

^cTotal metals; values in parentheses represent AB-DTPA-extractable metals.

Study 1

Pot Experiment. Seeds of spring wheat variety Auqab-2000 were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, whereas seeds of wheat variety Inqalab-91 were obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. A sandy clay loam soil spiked with 500 mg Pb kg⁻¹ was used for this experiment. Before filling the air-dried soil (11.5 kg) into experimental pots without any leaching provision, all the treatment soils were fertilized with 75 mg nitrogen (N) kg⁻¹ soil as urea, 100 mg phosphorus (P) kg⁻¹ soil as single superphosphate, and 70 mg potassium (K) kg⁻¹ soil as potassium chloride. Seven seeds were sown per pot, and five seedlings per pot were maintained to booting stage. The uprooted plants were crushed and mixed into the same pot. The second dose of fertilizer N at 75 mg N kg⁻¹ was applied 25 days after germination with irrigation water. The experiment was conducted in a wire house, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, which had an iron net-covered roof (the sides had only iron wire screen and no control of temperature and relative humidity). The wire house is located at 73° E, 31° N, at an altitude of 135 m above sea level. Ten days before harvesting, EDTA was applied at 0 (control) 2, 4, and 8 mmol kg⁻¹ dry soil, respectively, as solution on the surface of the pot soil. EDTA was used as disodium (Na₂)–EDTA salt (Merck) with a minimum assay of more than 99.5% and was obtained from Pakistan Scientific Stores, Faisalabad, Pakistan. Treatments were replicated three times in a two-factorial, completely randomized design; thus, 24 pots were used. Two h before harvesting, measurements of photosynthetic and transpiration rates were made on flag leaves of three randomly selected plants from each pot.

Aboveground tissues were harvested by cutting the stem 1 cm above the soil surface. Harvested plants were washed with 1% hydrochloric acid (HCl), tap water, and distilled water to remove any material adhering to plant parts. After blotting with tissue, fresh weights of shoots were recorded, and plant material was then air-dried for 2 days under paper covers in the shade. The air-dried plants were oven-dried at $65 \pm 5^\circ\text{C}$ for 72 h to constant weight. Plant material was ground to a fine powder in a mechanical grinder (MF 10 IKA, Werke, Germany) to pass through a 1-mm sieve. A 0.5-g portion of the plant sample was digested in a diacid mixture of nitric and perchloric acid (3:1) at 150°C (Miller 1998). The concentration of Pb in the shoot digest was determined with an atomic absorption spectrometer (AAS; Thermo Electron Corporation; Thermo Jarrell Ash, Franklin, Mass.).

The deionized water (DI)-extractable Pb content of soil samples was determined immediately after harvesting plants. Water-soluble metal content in soil of each pot was measured after extraction of 10 g of dry soil with 50 mL deionized water in an orbital shaker at 200 rpm for 16 h. After centrifugation, water extracts were filtered through Whatman No. 42 filter paper and analyzed for Pb with flame atomic absorption spectrometry (Santos et al. 2006).

Determination of Gas Exchange. Measurements of photosynthetic and transpiration rates were made using an open system LCA-4 portable infrared (IR) gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were made on flag leaves of three randomly selected plants from each pot. The determinations were made under the following conditions: leaf surface area 11.35 cm^2 , ambient carbon dioxide (CO_2) concentration (C_{ref}) $405.87\text{ }\mu\text{mol mol}^{-1}$, temperature of leaf chamber 31.8 to 38.5°C , chamber gas flow rate (V) 408 mL min^{-1} , molar flow of air per unit leaf area $251.43\text{ }\mu\text{mol s}^{-1}$, ambient pressure (P) 992 kPa , water vapor pressure in the chamber 21.2 – 24 mbar , and PAR (Q leaf) at leaf surface up to 1122 E .

Study 2

Leaching Column Experiment. A leaching column experiment was undertaken to monitor the leaching of Pb from the soil in response to different concentrations of the applied EDTA. Columns made of polyvinyl chloride (PVC) 40 cm long and 11 cm internal diameter were filled with soil incubated with Pb at 500 mg kg^{-1} . The lower end of each column was covered with plastic wire mesh and tightly held in place with thread and rubber bands. These columns were placed above plastic funnels mounted on iron stands, and receivers were placed beneath each funnel. Layers of 0.5 cm glass wool and 1.5 cm washed sand were initially spread at the base of each column to facilitate the leaching process, and subsequently air-dried soil (4.0 kg) was poured into each column and gently tapped to remove any air pockets. Two days later, each column was soaked with tap water equal to 100% of the soil saturation percentage and columns were allowed to equilibrate for 5 days while maintaining moisture content to obtain uniform packing. No crop was planted in these columns.

Columns were subjected to four leaching treatments: (1) tap water (control), (2) leaching solution containing EDTA at 2 mmol kg^{-1} soil, (3) leaching solution containing EDTA at 4 mmol kg^{-1} soil, and (4) leaching solution containing EDTA at 8 mmol kg^{-1} soil. The EDTA solution was applied on the surface of soil columns and a total of 4 pore volumes (PV) of water was applied in 1-PV installments. The treatments were replicated three times in a completely randomized design. Leachates were collected for infiltration

of each PV in plastic flasks affixed to the bottom of columns. The infiltration of 1 PV water continued for 1 to 5 days. The infiltrate was analyzed for Pb by AAS (Thermo S-Series).

Statistical Analysis

Results were analyzed using commercially available software MINITAB, version 14. The data were statistically evaluated by analysis of variance (ANOVA), least square means, and standard errors (SE). Differences were statistically significant at the $P = 0.05$ level.

RESULTS

Wheat Biomass and Tissue Water Contents (Study 1)

The shoot dry weight and tissue water contents are presented in Table 2. Statistical analysis showed that EDTA ($P < 0.001$), variety ($P < 0.001$), and EDTA \times variety ($P < 0.001$) effects were significant for shoot dry matter. Dry matter of Auqab-2000 increased with increasing rate of EDTA from 0 to 4 mmol kg⁻¹ but a further increase in the EDTA application rate (8 mmol kg⁻¹) resulted in a significant decrease. A different pattern of dry-matter production occurred in Inqalab-91, in which significantly more shoot dry matter was recorded for the control treatment than EDTA application. Although a slight decrease in dry-matter production of Inqalab-91 was recorded following the application of EDTA, there was no significant difference among the three rates of EDTA applied. Tissue water contents followed a pattern similar to that observed for shoot dry-matter yield.

Table 2
Effect of amendments on wheat crop biomass (g pot⁻¹) and tissue water content

	Shoot dry matter		Tissue water content	
EDTA	Auqab	Inqalab	Auqab	Inqalab
0	27.26 ± 0.43	30.54 ± 0.55	35.24 ± 2.30	50.94 ± 0.79
E-2	28.47 ± 0.49	29.80 ± 0.48	49.80 ± 2.16	47.71 ± 1.00
E-4	30.26 ± 0.32	29.92 ± 0.60	58.57 ± 4.35	50.28 ± 0.77
E-8	24.51 ± 0.69	29.75 ± 0.29	33.73 ± 1.43	49.36 ± 0.46
ANOVA				
EDTA				
<i>F</i> ratio	12.24		16.50	
<i>p</i> value	<0.001		<0.001	
Variety				
<i>F</i> ratio	45.28		13.16	
<i>p</i> value	<0.001		0.02	
EDTA and variety				
<i>F</i> ratio	11.70		18.17	
<i>p</i> value	<0.001		<0.001	

Note. E stand for EDTA and figures following the symbol are application rates of EDTA in mmol kg⁻¹.

Table 3
Effect of amendments on gas-exchange features of wheat varieties

	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	
	Auqab	Inqalab	Auqab	Inqalab
EDTA				
0	14.30 \pm 0.82	14.73 \pm 0.42	2.05 \pm 0.01	2.63 \pm 0.04
E-2	14.77 \pm 0.29	14.54 \pm 0.16	2.66 \pm 0.08	2.69 \pm 0.14
E-4	15.78 \pm 0.04	14.62 \pm 0.40	2.85 \pm 0.09	2.74 \pm 0.09
E-8	9.07 \pm 0.59	14.43 \pm 0.25	1.74 \pm 0.10	2.38 \pm 0.10
ANOVA				
EDTA				
<i>F</i> ratio	25.15		29.14	
<i>p</i> value	<0.001		<0.001	
Variety				
<i>F</i> ratio	12.78		21.09	
<i>p</i> value	0.003		<0.001	
EDTA and variety				
<i>F</i> ratio	22.35		9.29	
<i>p</i> value	<0.001		<0.001	

Note. E stand for EDTA and figures following the symbol are application rates of EDTA in mmol kg^{-1} .

Gas Exchange in Wheat Varieties

Photosynthetic and transpiration rates of the two wheat varieties studied are given in Table 3. Statistical analysis showed that EDTA ($P < 0.001$), variety ($P < 0.005$), and interaction (variety \times EDTA) effects were significant ($P < 0.001$) for both the gas-exchange attributes. The two wheat varieties responded differently to EDTA application. For Auqab-2000, increasing the rate of EDTA from 0 to 4 mmol kg^{-1} increased both the photosynthetic and transpiration rate, but application of EDTA at 8 mmol kg^{-1} significantly decreased these parameters. For Inqalab-91, photosynthetic and transpiration rates showed far less increase in adverse effects in the EDTA application rates.

Phytoextraction of Pb by Wheat Varieties

The concentration of Pb in shoots and total Pb uptake by wheat varieties with the application of EDTA is depicted in Table 4. Application of EDTA at 2, 4, and 8 mmol kg^{-1} significantly ($P < 0.001$) increased Pb concentration in shoots of both varieties. Although addition of EDTA to soil increased the concentration of Pb in shoots for both of the varieties, more Pb was accumulated by Auqab-2000 than by Inqalab-91. Application of EDTA at 8 mmol kg^{-1} resulted in 390.07 $\mu\text{g Pb g}^{-1}$ in shoots of Auqab-2000, which is 27 times more than that recorded with the control treatment. For wheat variety Inqalab-91, Pb concentration in shoots with the application of EDTA at the same rate (8 mmol kg^{-1}) resulted in 264.78 $\mu\text{g Pb g}^{-1}$ in shoots, which is only 17 times more than that recorded for the control treatment.

Table 4
Shoot concentration and uptake of Pb

	Pb concentration ($\mu\text{ g}^{-1}$)		Pb uptake (mg pot^{-1})	
EDTA	Auqab	Inqalab	Auqab	Inqalab
0	14.57 ± 0.62	15.24 ± 0.85	0.40 ± 0.02	0.47 ± 0.03
E-2	45.68 ± 1.62	44.16 ± 1.29	1.29 ± 0.03	1.31 ± 0.06
E-4	88.21 ± 1.95	79.73 ± 0.93	2.66 ± 0.05	2.38 ± 0.07
E-8	390.07 ± 8.08	264.78 ± 5.80	9.57 ± 0.46	7.86 ± 0.22
ANOVA				
EDTA				
<i>F</i> ratio	2986.60		810.32	
<i>p</i> value	<0.001		<0.001	
Variety				
<i>F</i> ratio	166.21		12.80	
<i>p</i> value	<0.001		0.003	
EDTA and variety				
<i>F</i> ratio	137.48		9.85	
<i>p</i> value	<0.001		<0.001	

Note. E stand for EDTA and figures following the symbol are application rates of EDTA in mmol kg^{-1} .

Total uptake of Pb by the two wheat varieties following the application of EDTA is presented in Table 4. Soil-applied EDTA at 2, 4, and 8 mmol kg^{-1} significantly ($P < 0.001$) increased Pb uptake by both the varieties. The Pb uptake into shoots of wheat varieties was related to treatment ($P < 0.001$), variety ($P = 0.003$), and interaction effects ($P = 0.001$). Wheat variety Auqab-2000 accumulated significantly more Pb in shoots than did Inqalab-91.

Effect of EDTA on Pb Extraction by Deionized Water

The changes in DI water-extractable Pb and soil pH with the application of EDTA are summarized in Table 5. In general, application of EDTA 10 days before harvesting enhanced deionized water-extractable Pb at the termination of experiment. Regarding the effect of soil-applied EDTA on Pb solubility, only treatment effects were significant ($P < 0.001$), but variety and interaction effects were nonsignificant. The DI water-extractable Pb reached a maximum mean Pb concentration of $244.83 \text{ mg kg}^{-1}$ with EDTA applied at 8 mmol kg^{-1} but a minimum of 3.93 mg kg^{-1} for the control treatment. However, the concentration of DI water-extractable Pb increased with an increase in the application rates of EDTA.

Environmental Risk of Pb after EDTA Application (Study 2)

The PV for soil columns of 40 cm long was 935 mL, and the leachate volume collected was almost similar to the PV. The analysis of leachates collected from control treatments and those receiving EDTA suggested that EDTA mobilized Pb, resulting in significant Pb leaching. The dynamics of Pb leaching from soils receiving EDTA are presented in Figure 1.

Table 5
Water-soluble Pb in response to EDTA application

EDTA	DI water Pb (mg kg ⁻¹)	
	Auqab	Inqalab
0	3.93 ± 0.26	4.33 ± 0.50
E-2	75.35 ± 3.04	76.69 ± 2.08
E-4	121.46 ± 7.09	115.80 ± 2.93
E-8	224.88 ± 16.33	217.88 ± 12.96
ANOVA		
EDTA		
<i>F</i> ratio	260.06	
<i>p</i> value	<0.001	
Variety		
<i>F</i> ratio	0.24	
<i>p</i> value	0.634	
EDTA and variety		
<i>F</i> ratio	0.140	
<i>p</i> value	0.935	

Note. E stand for EDTA and figures following the symbol are application rates of EDTA in mmol kg⁻¹.

The leachates from columns of control treatment contained very low Pb concentrations (Figure 1), but Pb concentrations were considerably higher in leachates from columns receiving EDTA. The results further demonstrated that Pb concentration in leachates was

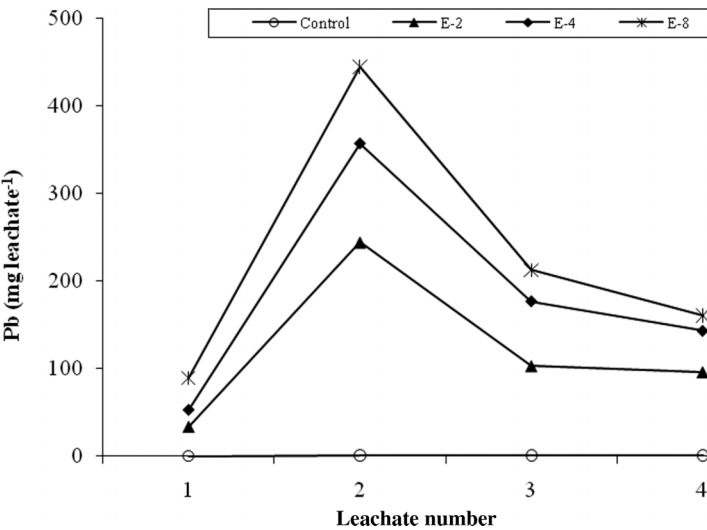


Figure 1. Concentration of Pb in leachates from sandy clay loam soil treated with EDTA. The means of three replicates are presented; error bars represent standard error of means. E stands for EDTA and figures following E are the rates of EDTA in mmol kg⁻¹.

positively related to application rates of EDTA. Maximum Pb concentration ($444.09 \text{ mg leachate}^{-1}$) was noted with the application of EDTA at 8 mmol kg^{-1} , and the minimum ($0.26 \text{ mg leachate}^{-1}$) was in leachates from the control treatment. Among leaching cycles, the most Pb was recovered in the second leachate, and Pb concentration decreased progressively in the third and fourth leaching cycles.

Discussion

As expected, soil-applied EDTA increased the concentration of Pb in soil solution over the control treatment (Table 5). The effect of EDTA on Pb solubilization was similar for both the wheat varieties. Increases of 57- and 50-fold over the control treatment were observed for water-soluble concentration of Pb with the application of $8 \text{ mmol EDTA kg}^{-1}$ soil in Auqab-2000 and Inqalab-91 sown soils, respectively. The ability of EDTA to enhance the release of Pb from insoluble or sparingly soluble compounds could be attributed to its higher binding capacity for Pb as reported previously (Blaylock et al. 1997; Huang et al. 1997; Wu, Hsu, and Cunningham 1999). Indeed, Shen et al. (2002) reported EDTA as the most efficient chelating agent for the release of soil-bound Pb, observing a 42-fold increase in soil solution Pb concentration 3 days after the application of EDTA at 1.5 mmol kg^{-1} soil over that for the control treatment.

The phytoextraction depends upon the metal concentration in shoots and high quantity of biomass produced (McGrath, Zhao, and Lombi 2002). The desired benefits of plant-based remediation technologies can never be achieved without increasing the tissue concentration of heavy metals, which in turn largely depends on the bioavailable amount (soluble, complexed, and chelated species) of heavy metals in soils. In spite of some reported success stories of increasing phytoavailability of heavy metals using EDTA, researchers have expressed concerns about EDTA-assisted phytoextraction because of the excessive levels of EDTA and heavy metals in soil solution resulting from the dissolution of soil-bound metals including Pb. Plants exposed to high levels of both the free Pb and free protonated EDTA produced a low biomass because of low seed germination, leaf wilt, chlorosis and necrosis, abscission, shoot desiccation, and reduced transpiration (Vassil et al. 1998; Lombi et al. 2001; Römkens et al. 2002; Grčman et al. 2001; Nascimento, Amarasiriwardena, and Xing 2006; Saifullah, Ghafoor, and Qadir 2009). Reduction in aboveground biomass could lead to a decrease in the total amount of metals removed by plants (Quiroz, Garcia, and Ilangovan 2002). In our study, with the addition of chelating agent (EDTA), shoot dry matter of both the wheat varieties remained less than in their respective control plants. Application of EDTA, especially at 8 mmol kg^{-1} , significantly decreased shoot dry matter of Auqab-2000 compared to that of Inqalab-91. Without EDTA application, shoot dry matter was 27.26 and 30.54 g pot^{-1} for Auqab-2000 and Inqalab-91, respectively. However with the application of EDTA at 8 mmol kg^{-1} , shoot dry matter of Auqab-2000 and Inqalab-91 was decreased to 24.51 and 29.75 g pot^{-1} , respectively. In our study, the addition of EDTA to soil had an almost similar effect on Pb solubility in soils planted to both Auqab-2000 and Inqalab-91. However, reduction in shoot dry matter was more for Auqab-2000 than that for Inqalab-91, which could be attributed to greater tolerance of Inqalab-91 to higher concentrations of EDTA and/or Pb than the other variety.

Gas-exchange attributes (photosynthetic and transpiration rates) were determined 10 days after application of EDTA to the soil. Application of EDTA significantly decreased the photosynthetic and transpiration rates (Table 3), but both the tested varieties had similar photosynthetic and transpiration rates. Application of EDTA up to 4 mmol kg^{-1} slightly increased photosynthetic and transpiration rates in Auqab-2000; however, EDTA

at 8 mmol kg⁻¹ decreased both the parameters. A different pattern emerged with respect to gas-exchange attributes in Inqalab-91. Photosynthetic and transpiration rates of Inqalab-91 decreased with the application of EDTA, but the decrease was very small. The shoot dry matter and gas-exchange data confirmed that Inqalab-91 is more tolerant than Auqab-2000.

Compared to control soils, application of EDTA at 2, 4, and 8 mmol kg⁻¹ increased the concentration of Pb in shoots of wheat variety Auqab-2000 by 3, 6, and 26-fold, respectively. For Inqalab-91, the corresponding increases were 2.89-, 5.23-, and 17.23-fold, respectively, over that for the shoots of control plants. An increase in uptake of Pb, induced by the application of EDTA, could be explained by its effect on enhancing the solubility of Pb (Table 5) and absorption of Pb-EDTA complex by plants (Vassil et al. 1998; Luo et al. 2006a). In the current studies, application of EDTA at 8 mmol kg⁻¹ caused a reduction in plant dry matter and gas-exchange attributes only in Auqab-2000, but not in Inqalab-91. Therefore, greater accumulation of Pb by wheat variety Auqab-2000 could be attributed to its lower tolerance to higher EDTA as well as Pb levels in soil that caused damage to physiological barriers in roots (Vassil et al. 1998), which otherwise could control the uptake of solutes. Recently, Gregorio et al. (2006) reported significantly enhanced uptake of Pb by *Brassica juncea* with the combined application of Triton X-100 (a nonionic surfactant) and EDTA compared with plants treated with EDTA alone. This enhanced uptake of Pb with the combined application of EDTA and Triton X-100 was attributed to phytotoxic effects of Triton X-100 on roots, resulting in an increased permeability of roots to EDTA-Pb. Similarly, Ochiai and Matoh (2002) reported that excessive Na⁺ in rooting medium could destroy root structure in rice, resulting in enhanced flow of Na⁺ into xylem vessels and increased accumulation of Na⁺ in shoots. Despite less shoot dry matter, Auqab-2000 extracted more Pb from soil than did Inqalab-91, indicating that the effects of EDTA on shoot metal concentration were more important than effects on yield for determining metal removal.

In this study, we measured transpiration rates to determine its effects on Pb accumulation in plant shoots. It was observed that soil-applied EDTA at 8 mmol kg⁻¹ caused depression in transpiration rates of both the wheat varieties. Greater depression in transpiration rate was observed for Auqab-2000 than for Inqalab-91. Despite its lower transpiration rate, Auqab-2000 accumulated more Pb than Inqalab-91. Therefore, this study indicated that transpiration rate did not appear to be a critical factor for the phytoaccumulation of Pb. Similar results have been reported by Epstein et al. (1999), who concluded that Pb accumulation by *Brassica juncea* was not dependent upon transpiration rate. As suggested by previous researchers (Epstein et al. 1999; Collins et al. 2002), however, it could be assumed that most of the Pb had already been accumulated before the onset of adverse effects of EDTA and/or Pb become measurable on transpiration rate.

The potential for offsite migration of heavy metals resulting in the contamination of groundwater is one of the major issues associated with EDTA-assisted phytoremediation. In leachates from the control columns (study 2), concentration of Pb was very low, indicating that a small amount of Pb has been removed with distilled water. For columns receiving DI water, this low concentration was only 0.25% of the initial total Pb in soil (Table 6). The corresponding values for Pb leaching with EDTA applied at 2, 4, and 8 mmol kg⁻¹ soil were 27.41, 41.96, and 52.11% of the initial Pb in soil; that is, there was a gradual increase in the Pb leaching with EDTA application rates. Several researchers have reported a positive relationship between EDTA application rate and metal removal from soils (Grčman et al. 2003; Wenzel et al. 2003). Because soil-applied EDTA has known effects on solubilization of insoluble Pb fractions (Saifullah et al. 2008; Saifullah, Ghafoor, and Qadir 2009) followed by its enhanced movement with infiltrating soil solution (Chen, Li, and Shen 2004), in addition to enhanced EDTA-complexed Pb absorption by plants (Ruley et al.

Table 6

Lead leached (mg leachate^{-1}) from columns in response to application of various rates of EDTA and control treatment

Treatment	L1	L2	L3	L4	Lead leached (Total)
Control	0.3(0.01)	1.4(0.08)	1.2(0.07)	1.4(0.08)	4.3(0.25)
E-2	33.6(1.93)	244.4(14.04)	103.0(5.92)	96.0(5.52)	477.0(27.41)
E-4	53.4(3.07)	356.6(20.49)	176.9(10.17)	143.2(8.23)	730.2(41.96)
E-8	89.6(5.15)	444.1(25.52)	215.5(12.21)	160.6(9.23)	909.8(52.11)

Notes. Values are means of three replicates, and values between parentheses are the percentage of Pb leached from columns (total Pb at the start of experiment in each columns was 1740 mg Pb). L stands for leachate, and figures following L are the numbers of leachate cycles. E stands for EDTA, and figures following the symbol are application rates of EDTA in mmol kg^{-1} . Soil used in columns was sandy clay loam in texture.

2006; Tandy et al. 2006), the amount of Pb passing through contaminated soils increased with increasing rate of EDTA application (Saifullah et al. 2008). However, the soil column height in the present studies was 40 cm, and still chances of Pb leaching from longer soil columns or in soils remain evident, resulting from less degradation and adsorption of EDTA or Pb complexed with EDTA (Wu et al. 2003). There was considerable increase in Pb leaching (25.52% of initial soil Pb) with the second PV of applied distilled water, which decreased gradually to 12.21 and 9.23% of the initial soil Pb (Table 6). Indirectly, this pattern points towards another issue: Pb leached with the second PV could move to deeper soil layers with the following irrigations owing to high water potential of soil solutions with the later leaching cycles (Wu et al. 2004). Such increased movement of Pb through soil could be presumed to contaminate groundwater, which may become a serious environment concern, especially for areas receiving high rainfall or raw sewage for irrigation (Wenzel et al. 2003). Hence, EDTA treatment of contaminated soils must be practiced very carefully, as has already been reported in literature (Evangelou, Ebel, and Schaeffer 2007; Meers et al. 2008). The Pb concentration in leachates was strongly related to the rate of EDTA applied (Figure 2). From columns treated with 2, 4, and 8 mmol kg^{-1} EDTA, 27.41, 41.96, and 52.11% of the initial total Pb was leached with just 4 PV of water. Several researchers have reported a positive relationship between EDTA application rate and metal removal from soils (Grčman et al. 2003; Wu et al. 2004).

Conclusions

The results from present study indicate that wheat variety Auqab-2000 exhibited less shoot biomass, photosynthetic rate, and transpiration rate than those from Inqalab-91. Despite having similar concentrations of water-soluble Pb in soil growing with both varieties, larger accumulation of Pb occurred in shoots of Auqab-2000 than in shoots of Inqalab-91. Further studies are needed to isolate the differences in uptake mechanisms in different varieties of the same species during EDTA-assisted phytoextraction. High concentrations of Pb were found in leachates received from columns treated with EDTA. These results suggest that EDTA addition for enhancing soil cleanup must be designed properly to minimize the uncontrolled release of metals from soils into groundwater.

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